development through the regulation of morphogenesis, instead of cell fate specification.

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Program/Abstract # 126
Visualizing morphogen distribution in lumen
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Diffusion dynamics of extracellular signalling molecules have been extensively studied recently. In several developing systems such as neural tube, it has been suggested that morphogen in lumen i.e. inside neural tube, may play an important role during development. However, the detection of diffusing molecule is technically difficult because the molecule must be immobilized by fixation to be visualized by immunohistochemistry. In the case of lumen, extracellular matrix is lacking which can trap diffusing signalling molecule, so distribution of diffusing molecule cannot be detected with conventional immunohistochemistry. In the present study, we developed a simple method to visualize the distribution of diffusive signalling molecule in lumen. We validated the result by collecting the liquid in lumen and detecting the molecule by Western blot. Then we applied the method to several developing organs to examine the role of the signalling molecule in lumen during development.

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Program/Abstract # 127
Shh signaling regulates reciprocal epithelial–mesenchymal interactions controlling palate development
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The mammalian secondary palate arises by outgrowth from the oral sides of the paired maxillary processes flanking the primitive oral cavity. The outgrowth of the bilateral palatal shelves depends on reciprocal interactions between the oral ectoderm and the underlying neural crest derived mesenchyme. Previous studies have implicated Sonic hedgehog (Shh) as an important epithelial signal for regulating palatal growth. However, the cellular and molecular mechanisms through which Shh regulates palatal development in vivo have not been directly analyzed, due in part to early embryonic lethality of mice lacking Shh or other essential components of the Shh signaling pathway. Using Cre/loxP-mediated tissue-specific inactivation, in either the developing palatal epithelium or palatal mesenchyme, of the *Smoothened* (*Smo*) gene, we show that the epithelially expressed Shh signals to the palatal mesenchyme to regulate *cyclin-D1* expression and palatal mesenchyme cell proliferation. In addition, Shh signaling maintains *Fgf10* mRNA expression in the palatal mesenchyme and secondarily affects palatal epithelial cell proliferation. Together with previous reports that the mesenchymally expressed Fgf10 signals to the palatal epithelium to positively regulate *Shh* mRNA expression, these data demonstrate that Shh and Fgf10 function in a positive feedback loop mediating the reciprocal epithelial–mesenchymal interactions that regulate palatal outgrowth.

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Program/Abstract # 128
Wnt2 signaling regulates morphogenesis of the inflow tract and atrioventricular canal during cardiac development
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The central role of Wnt ligands in early cardiac commitment, expansion, and differentiation is poorly understood. Wnt2a is expressed in precardiac mesoderm and later in the inflow portion of the heart including the atria. Our Wnt2a mutants have more than 80% embryonic and perinatal lethality. Morphological defects are observed in the development of the atrioventricular canal, endocardial valves and in the atrial and ventricular myocardium. Loss of Wnt2a signaling leads to decreased sarcomere development in the myocardium of Wnt2a mutant heart.Wnt2b is expressed in a similar pattern as Wnt2a in cardiac mesoderm. Of note, Wnt2b null mice are viable and do not display obvious cardiac phenotypes. Remarkably, Wnt2a/2b DKO mutants die by E14.5 and display more severe defects in AV canal, endocardial cushion, and atrial myocardium development than Wnt2a single mutants. Microarray studies have indicated that endocardial marker genes are significantly upregulated, whereas myocardial marker genes are significantly downregulated in Wnt2a mutant hearts. Two cardiac-specific transcription factors, GATA6 and Sall3, are markedly reduced in Wnt2a mutant hearts, providing insight into possible mechanisms underlying the phenotype in Wnt2a mutants. The expression of Isl1 is significantly downregulated in the atrium of Wnt2a mutant hearts. These data implicate that Wnt2 regulates differentiation of cardiac precursors into myocardial and endocardial lineages, which is required for proper AV canal morphogenesis, endocardial valve formation, and atrium development.

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Program/Abstract # 129
Daam1 is required for mouse heart morphogenesis
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Daam1 is a formin-homology protein implicated in b-catenin independent Wnt signaling. Daam1 complexes with the Wnt effector protein Disheveled and Rho-family GTPases in the presence of Wnt receptor binding and is required for Wnt proteins to activate RhoA. Daam1 is highly expressed in cardiac myocytes during mouse heart development. We therefore deleted Daam1 specifically in the heart to determine the role that Daam1 plays during heart morphogenesis. Daam1 mutant hearts have greatly enlarged right atria relative to the hearts of wild type siblings at all ages examined. Furthermore, histological examination reveals the presence of atrial septal defects in Daam1 mutant hearts and an expansion of atrial tissue around proximal portions of the incoming vena cava. Finally, the myocardium of Daam1 mutants has a disrupted cellular architecture in both the atrial and ventricular compartments and transmission electron microscopy reveals defects in the intercalated discs of Daam1 mutants. To further examine the role that Daam1 plays in cell–cell adhesion, we transfected primary cultures of neonatal ventricular myocytes with either Daam1 or control siRNA. In control treated cultures, beating foci of cardiac myocytes are highly interconnected to one another by cellular protrusions but these protrusions are often thin or incomplete in cultures treated with Daam1 siRNA.

Interestingly, cardiac myocytes transfected with Daam1 siRNA contract at a rate approximately three times higher than control treated myocytes, suggesting that Daam1 regulates cardiac contractility. Thus, Daam1 regulates cardiomyocyte cell–cell interactions and may be required for late stage remodeling in the heart.

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Program/Abstract # 130
LR asymmetric morphogenesis of heart looping
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Establishment of left right (LR) axis formation is required for correct positioning and function of internal organs. Our long-term goal is to reveal the mechanism of how LR asymmetric signals regulate LR asymmetric morphogenesis. Our previous work has revealed the significance of the Nodal-signaling pathway in the establishment of the LR asymmetry, which is required for the correct positioning and morphogenesis of internal organs. However, the cellular and molecular mechanisms by which LR signals bring about asymmetric organ development are unknown. We are investigating how LR signals regulate cell behaviors in asymmetric heart looping morphogenesis. The heart is the first organ to exhibit LR asymmetry, and its looping morphogenesis is readily accessible in culture. The left and right primordial heart fields migrate and fuse to form a single heart tube that subsequently loops rightward. Looping morphogenesis is achieved by rotation of the heart tube exerted by the out flow tract rotation and overriding of left caudal rudiments. We focus on cellular behaviors in these events and have analyzed cell movement by dye labeling and morphological changes by time lapse imaging, cell proliferation, and gene expression in the chick and mouse. Our cell tracing experiments showed changes in cell positions from mediolateral to anteroposterior orientation, and significant cell cluster extension during migration, which could be major forces for looping morphogenesis. Based on these data, we will discuss how LR signals regulate LR asymmetric looping of the heart.

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Program/Abstract # 131
Inturned PCP effector gene is required for cilia biogenesis and mouse embryonic development
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Cilia are cell surface organelles required for mammalian embryonic development and multiple adult physiological functions. Recent protein localization studies indicated that some proteins regulating the planar cell polarity (PCP) pathway are localized to the axonemes and basal bodies of the primary cilia. However, the functional significance of this connection between cilia and PCP regulation has yet to be corroborated in mammals. The inturned PCP effector (Intu) gene was originally identified in the fruit flies based on its role in regulating the formation and polarity of wing hairs. In the current study, we take both forward and reverse genetic approaches to study the function of Intu in the mouse. Double-thumb (Dtm), a hypomorphic mutant allele of Intu generated by chemical mutagenesis, exhibits polydactyly and behavioral defects including circling and head bobbing. We also generated a null Intu mutant through gene-targeting in mouse embryonic stem (ES) cells, and found that the complete loss of Intu function results in multiple developmental defects including neural tube defects, spinal cord patterning defects and severe polydactyly. Our scanning electron microscopic study indicated that cilia biogenesis is disrupted by the mutation in Intu. In conclusion, our study provided the first evidence in the mouse that a PCP effector gene is required for ciliogenesis and embryonic development.

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Program/Abstract # 132
Patterning of the mouse embryonic germ layers: The Townes and Holtfreter cell sorting experiments revisited
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The experiments undertaken by Townes and Holtfreter described that cells dissociated from the embryonic germ layers segregated homotypically once homogeneously mixed. Subsequently, Steinberg pioneered the differential adhesive hypothesis (DAH) to explain these and other patterning phenomena. We have revisited these issues using embryoid bodies derived from mouse embryonic stem (ES) cells where nascent endoderm is distributed, initially internally but eventually sorts to the spheroid surface. Wild type and E-cadherin null ES cells were used to generate chimeric embryoid bodies to probe the relative importance of adhesion and differentiation for the partitioning of endoderm to surface. When undifferentiated wild type and undifferentiated E-cadherin null ES cells were mixed, the resulting cell aggregates consisted of a core of highly adhesive wild type cells surrounded by E-cadherin null cells, consistent with the DAH. Both ES cell types were also differentiated into primitive endoderm-like cells by exposure to retinoic acid and then mixed with undifferentiated counterparts. We observed that endoderm cells always sorted to the surface to form an endoderm layer irrespective of their E-cadherin status or that of their undifferentiated counterparts. Thus, the sorting of primitive endoderm from pluripotent ES cells contradicts the DAH. We propose that the autonomous ability of endoderm cells to generate apical polarity, rather than differential adhesive affinity, governs the developmental restriction of primitive endoderm cells to a superficial layer.

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Program/Abstract # 133
Sequential roles of Wnt signaling/β-catenin in mouse ventral dermal development
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